

In the Specification:

Please amend the specification as shown:

Please delete the paragraphs on page 48, line 13 to page 49, line 19 and replace them with the following paragraphs:

GSK-3 β kinase assay

GSK-3 was obtained from New England Biolabs (UK) Ltd. , Hitchin, Herts. The recombinant enzyme was isolated from a strain of *E. coli* that carries a clone expressing GSK-3 β derived from a rabbit skeletal muscle cDNA library [34]. Inhibition of GSK-3 function was assessed by measurement of phosphorylation of CREB phosphopeptide KRREILSRRPpSYR (**SEQ ID NO: 1**) in the presence of test compounds. Using a 96-well assay format, GSK3 (7.5 U) was incubated for 30 min at 30 °C in a total volume of 25 μ L in 20 mM MOPS pH 7.2, 25 mM β -glycerophosphate, 5 mM EGTA, 1 mM DTT, 1 mM Na₃VO₃, 40, μ M CREB peptide, 15 mM MgCl₂ and 100, μ M ATP (containing 0.25 μ Ci [γ -³²P]-ATP) in the presence of varying concentrations of test compound. The samples were transferred to 96-well p81 filter plates (Whatman Polyfiltrronics, Kent, UK), and the plates were washed 4 times with 200 μ L/well of 75 mM aq orthophosphoric acid. Scintillation liquid (50 μ L) was added to each well, and incorporated radioactivity for each sample was determined using a scintillation counter (TopCount, Packard Instruments, Pangbourne, Berks, UK).

CDK/cyclin kinase assays

Compounds were investigated for their CDK2/cyclin E, CDK2/cyclin A, CDK1/cyclin B, and CDK4/cyclin D1 inhibitory activity. His₆-tagged (**SEQ ID NO: 2**) recombinant human cyclin-dependent kinases CDK1/cyclin B1, CDK2/cyclin E, CDK2/cyclin A, and CDK4 were expressed in sf9 insect cells using a baculovirus expression system. Recombinant cyclin D1 was expressed in *E. coli*. Proteins were purified by metal chelate affinity chromatography to greater than 90 % homogeneity. Kinase assays were performed in 96-well plates using recombinant CDK/cyclins. Assays were performed in assay buffer (25 mM β -glycerophosphate, 20 mM MOPS, 5 mM EGTA, 1 mM DTT, 1 mM Na₃VO₃, pH 7.4), into which were added 2-4 μ g of active enzyme with appropriate substrates (purified histone H1 for CDK1 and CDK2, recombinant GST-retinoblastoma protein (residues 773-928) for CDK4). The reaction was initiated by addition of Mg/ATP mix (15 mM MgCl₂ + 100 μ M ATP

Express Mail Label No. EV 957643497 US
U.S. Serial No.10/588,372
Atty. Dkt. No. CCI-068US

with 30-50 kBq per well of [γ -³²P]-ATP) and mixtures incubated for 10-45 min, as required, at 30 °C. Reactions were stopped on ice, followed by filtration through p81 or GF/C filterplates (for CDK4) (Whatman Polyfiltrronics, Kent, UK). After washing 3 times with 75 mM aq orthophosphoric acid, plates were dried, scintillant added and incorporated radioactivity measured in a scintillation counter (TopCount, Packard Instruments, Pangbourne, Berks, UK). Compounds for kinase assays were made up as 10 mM stocks in DMSO and diluted into 10 % DMSO in assay buffer. Data was analysed using curve-fitting software (GraphPad Prism version 3.00 for Windows, GraphPad Software, San Diego California USA) to determine IC₅₀ values (concentration of test compound which inhibits kinase activity by 50 %).